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Running head: Arsenic in drinking water and diabetes in Denmark

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Abstract

Background: Established causes of diabetes do not fully explain the epidemic. High level arsenic exposure has been implicated in diabetes risk but the effect of low-level arsenic exposure in drinking water remains unclear.

Objective: To determine if long-term exposure to low-level arsenic in drinking water in Denmark is associated with increased risk of diabetes using a large prospective cohort.

Methods: During 1993-1997 we recruited 57,053 persons. We followed each cohort member for diabetes occurrence from enrollment until 31 December 2006. We traced and geocoded residential addresses of the cohort members and used a geographic information system to link addresses with water supply areas. We estimated individual exposure to arsenic using all addresses from 1 January 1971 until the censoring date. Cox proportional hazards models were used to model the association between arsenic exposure and diabetes incidence, separately for two definitions of diabetes: all cases and a more strict definition, where cases of diabetes based solely on blood glucose results were excluded.

Results: Over a mean follow-up of 9.7 years of 52,931 eligible subjects, there were 4,304 (8.1%) diabetes cases in total, and 3,035 (5.8%) cases of diabetes based on a stricter definition. The adjusted incidence rate ratio's per 1 μg/L increment in arsenic levels in drinking water were (IRR = 1.03; 95% CI: 1.01, 1.06) and (IRR = 1.02; 95% CI: 0.99, 1.05) for all and strict diabetes cases, respectively.

Conclusions: Long-term exposure to low-level arsenic in drinking water may contribute to development of diabetes.

Introduction

The prevalence and incidence of diabetes is rapidly increasing in all countries including Denmark, presenting a major public health threat (Carstensen et al. 2008; Danaei et al. 2011; WHO 2011). Established risk factors are mainly related to lifestyle and include older aged populations, obesity and physical inactivity and in part related to a family history of diabetes and genetic polymorphisms. But these factors do not fully explain the epidemic. Given that almost 400 million persons are diagnosed with diabetes worldwide (WHO 2011) and the severe long term consequences of this disease in terms of morbidity, mortality and economic costs, there is an increased need to understand the effects of non-traditional risk factors such as environmental chemicals.

Arsenic occurs in both organic and inorganic environmental forms (Eyre et al. 2004; Mandal and Suzuki 2002). Organic arsenic, is found primarily in food, whereas inorganic arsenic is mostly found in aquifers (Eyre et al. 2004; Mandal and Suzuki 2002), where it accumulates by natural processes such as weathering and erosion (Smedley 2008). Globally, exposure to inorganic arsenic via groundwater used for drinking is associated with most health risks. In Denmark all drinking water from tap water is derived from groundwater (DANVA 2010), this tap-water is very clean and not chlorinated and of bottle water quality at the tap (Thomsen et al. 2004). It is the standard in Denmark to use tap water for cooking, coffee, tea and drinking. Thus the consequences of a possible relationship between low-level groundwater arsenic exposure and population health are serious.

Arsenic exposure has been implicated in the diabetes epidemic. Mechanisms remain unclear but based on *in-vitro* studies, they are thought to include disruption of several pathways related to

pancreatic β-cell function and insulin sensitivity, including oxidative stress, glucose uptake and transport, gluconeogenesis, adipocyte differentiation and Ca^{2+} signaling (Diaz-Villasenor et al. 2007; Druwe and Vaillancourt 2010; Tseng 2004). Two recent systematic reviews and a meta-analysis of epidemiological studies addressing the association between arsenic exposure in drinking water and diabetes risk conclude that the positive association with high concentrations of inorganic arsenic exposure was consistent, but also concludes that the evidence regarding low-level exposure, defined as below 50 ppb (equivalent to 50 μg/L), remains unclear and that a threshold might exist (Maull et al. 2012; Navas-Acien et al. 2006; Wang et al. 2013). The role of low-level arsenic in diabetes risk needs to be elucidated and the need for future research including large prospective studies in areas of low arsenic exposure using individual arsenic exposures has been recommended (Maull et al. 2012).

The Diet, Cancer and Health cohort is a large prospective study with detailed information on potential confounders collected at baseline and the Danish National Diabetes Register (Carstensen et al. 2008; Carstensen et al. 2011) enables objective ascertainment of diabetes on a national scale. By combining geo-coded past and present residential addresses of cohort participants, obtained from the Danish Civil Registration System (CRS) (Pedersen 2011), with geographic information on water supply areas, the estimation of individual arsenic exposure of all cohort participants was possible.

The purpose of this large population based prospective study was to determine if individual long-term exposure to low-level inorganic arsenic in drinking water is associated with increased risk of diabetes.

Methods

The Diet, Cancer and Health Cohort

The present prospective cohort study was based on the prospective Diet, Cancer and Health cohort consisting of 57,053 participants, aged 50 to 64 years, enrolled in 1993-1997 (Tjønneland et al. 2007). The participants had to be born in Denmark, live in the Copenhagen or Aarhus areas at the time of enrollment, and be without a cancer diagnosis registered in the Danish Cancer Registry (Tjønneland et al. 2007). The baseline examination included a self-administered, interviewer checked, questionnaire on diet, beverages, present and previous smoking habits, length of school attendance, as well as other items related to health, lifestyle and socio-economic status (Overvad et al. 1991; Tjønneland et al. 1991; Tjønneland et al. 2007). Height, weight and waist circumference were measured by trained staff according to standardized protocols. The study was approved by the Scientific Ethics Committee for Copenhagen and Frederiksberg and The Danish Data Protection Agency and written informed consent was obtained from all participants prior to enrollment.

Since establishment of the CRS (Pedersen 2011) in 1968, all citizens of Denmark have been given a unique personal identification number, which allows accurate linkage between registers. We used the CRS to obtain information on date of death, emigration or disappearance of the cohort members and information on past and present residential addresses.

The Danish National Diabetes Register

Using the personal identification number we also linked the cohort members to the Danish National Diabetes Register (NDR) to identify incident diabetes cases between baseline (1993-1997) and either death, emigration, disappearance or end of follow-up (31 December 2006).

The NDR was established in 2006 by the National Board of Health to describe and monitor the occurrence of diabetes in Denmark and provide data for epidemiological research. Establishment of this register has been previously discussed in detail (Carstensen et al. 2008; Carstensen et al. 2011). In brief NDR links three existing nationwide administrative records in the Danish health care system. Namely, the *National Patient Register* (Lynge et al. 2011), containing hospitals and outpatient clinics discharge diagnoses since 1994; the *National Health Services Register* (Andersen et al. 2011), with information of all services provided by general and specialist practitioners since 1973 and the *National Prescription Registry* (Kildemoes et al. 2011) containing all prescriptions dispensed at Danish pharmacies since 1993 (Carstensen et al. 2011).

Inclusion criteria for the NDR were one or more of the following: i) a diabetes hospital discharge diagnosis in the *National Patient Register* (Lynge et al. 2011) defined as ICD10 (WHO 2010): E10-14, H36.0, O24 (excluding DO24.4), or ICD8: 249, 250; ii) record of chiropody for diabetic patients in the *National Health Services Register* (Andersen et al. 2011), iii) record of five or more blood glucose measurements within 1 year or two blood glucose measurements per year in five consecutive years in the *National Health Service Register* (Andersen et al. 2011); iv) record of a second purchase of insulin or second purchase of oral glucose-lowering drugs within 6 months, registered in the *National Prescription Registry* (Kildemoes et al. 2011). At least two inclusion criteria were met by 60% of all patients in NDR and 47% met at least three criteria (Carstensen et al. 2008). Due to different dates of initiation of the underlying registers and accumulation of prevalent cases, only incidence values after 1 January 1995 were found to be reliable (Carstensen et al. 2008). Thus, the incidence of diabetes was defined as the earliest record in the diabetes register occurring after 1 January 1995, between baseline (1993-1997) and 31 December 2006. In addition to this original NDR definition (all diabetes), we also defined a

more strict definition of incidence (strict diabetes), by excluding persons who were included to NDR solely due to a blood glucose test, as a number of these people may not have diabetes. We have previously used this register and these two definitions to assess associations between exposure to air pollution and risk of developing diabetes (Andersen et al. 2012).

Residential histories

Using the unique personal identification number of the cohort members, we traced residential histories in CRS between 1971 and 2006. Each residential address contained a unique identification code composed of a municipality-, road- and house number code. The dates the persons had moved to and from each address were noted. The addresses were then linked to a database of all official addresses and their geographical co-ordinates in Denmark. Geographical co-ordinates were obtained for 98% of all the addresses the cohort members had lived in.

Water supply and arsenic exposure

The methods of obtaining arsenic concentrations in Danish drinking water for the cohort participants has been previously described in detail (Baastrup et al. 2008). In brief, arsenic concentrations were obtained from a database managed by the Geological Survey of Denmark and Greenland (MCEB 2014), which is the most important national source of information in Denmark on the conditions of groundwater, aquifers and tap-water sent to the consumers (Thomsen et al. 2004). Different analytical methods were used for measuring arsenic in Danish drinking water throughout the investigated period relevant for our study. But the majority of the analyses were performed with Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and ICP-atomic emission spectroscopy. We assumed a detection limit for these methods between 0.03 and 0.1 µg/l. The spatial locations of water utilities were determined by their geographical

co-ordinates, also registered in that database. Average arsenic concentrations for each utility were calculated based on the 4,954 compulsory measurements taken at the outlet water pipe sending tap water to consumers in 2,487 water utilities from 1987 to 2004. This average was assumed to be representative of the arsenic concentrations throughout the study period 1971-2006. In the geographical areas most densely populated with study participants, we mapped the geographical areas supplied by each water supply unit, thus covering 76% of addresses, which enabled linkage of each address to the exact water supply unit delivering drinking water to the household. For the remaining 24% of addresses, the "nearest water supply unit approach" was applied. Volume-weighted arsenic concentrations were calculated for the areas receiving water from more than one utility. Using ArcMap 10.1 (ESRI) we linked the geo-coded cohort addresses with water supply areas or water utilities and their associated arsenic concentrations (Baastrup et al. 2008).

Time-weighted average arsenic exposure was calculated as the arsenic concentration in drinking water multiplied by time lived at each address, summed for all addresses lived at during the study period and divided by total observation time, with the unit micrograms per liter.

Statistical methods

The analyses were based on a Cox proportional hazards model with age as the underlying time scale ensuring that risk estimates were based on individuals at exactly the same age (Thiebaut and Benichou 2004). We used left truncation at age of recruitment, so that people were considered at risk from enrollment into the cohort, and right censoring at the age of diabetes (event), death, emigration, disappearance or end of follow-up on 31 December 2006, whichever came first, separately for the two definitions of diabetes. People diagnosed with diabetes prior to enrolment were excluded from the analyses.

Exposure, expressed as time-weighted average arsenic in water since 1 January 1971 was entered into the statistical diabetes risk models as a time-dependent variable; thus recalculating exposure for non-censored persons at the time of each censor. The effects of arsenic exposure on diabetes was evaluated in several steps with adjustment for *a-priori* defined confounders: (1) adjusted only for age; and (2) also including calendar year to account for time trends in diabetes incidence over follow-up time; and full adjustment for recognized diabetes risk factors: sex, body mass index (BMI), waist circumference (cm), smoking status, environmental tobacco smoke (ETS), leisure time physical activity, alcohol intake, fruit intake, vegetable intake, saturated fat intake, educational level and socioeconomic status.

ETS indicates living with a smoker and or/exposure to second-hand smoke at work for minimum 4 hours/day. Socio-economic status was based on municipality/district information on education, work market affiliation and income of the municipality/district each cohort participant had lived in at the time of enrollment. Data on individual dietary intake of vegetables, fruit and other foods were obtained from detailed self-administered, interviewer-checked, food-frequency questionnaires. The dietary questions were designed to collect information about dietary habits, participants were asked how often on average they had consumed the different types of foods during the preceding 12 months. The frequency of consumption was categorized into 12 groups ranging from never to 8 or more times a day. A mean daily intake of foods (g/day) was calculated by multiplying the frequencies of intake by a gender-specific portion size using the software program FoodCalc Version 1.3 (Lauritsen 2004).

We formed four intervals for arsenic exposure using the 25^{th}, 50^{th} and 75^{th} percentiles for time-weighted average arsenic since 1971 for all participants as the cut-off points and estimated the

incidence rate ratios (IRRs) for the higher exposure ranges compared with the lowest exposure range. IRRs were also estimated as linear trends in arsenic concentrations.

Potential modifiers of an effect between arsenic exposure and diabetes included waist circumference, length of education, smoking status and leisure time physical activity (cycling, walking and sport) as well as co-morbid conditions (cardiovascular disease including myocardial infarction and/or stroke at enrollment); effect modification was evaluated by introducing interaction terms into the adjusted model and using the Wald's test. Since arsenic concentrations were generally higher for persons enrolled in the Aarhus area than those in the Copenhagen area, we included separate analyses of risk in models stratified by enrollment area, knowing that results of such stratifications are limited as subjects moved residences throughout the study period, such that 11% and 14% lived outside the Copenhagen and Aarhus in 1971 and at the end of follow-up, respectively.

The results are expressed as IRRs with two-sided 95% confidence intervals (CIs) on the basis of the Wald test statistic for regression parameters in SAS (version 9.2; SAS Institute, Cary, NC), whereas exposure-response curves with 95% confidence limits were visualized using a restricted cubic spline in R (library Survival and Design, version 2.13.1) (Harrel 2001).

Results

Among the 57,053 cohort members, we excluded 571 due to a cancer diagnosis before enrollment; 2 with an uncertain date of cancer diagnosis; 1,191 due to either a self-reported diabetes before enrollment, a diabetes record in NDR before baseline or with a diabetes diagnosis from NDR between baseline and 1 January 1995; 960 for which address history was not available in the CRS or their baseline address could not be geo-coded and 1,398 because of

missing data in potential confounders or arsenic exposure information. The eligible 52,931 participants had lived in a total of 135,601 addresses and were followed up for diabetes for an average of 9.7 years. We identified 4,304 (8.1%) cases of diabetes in total (incidence rate 8.4 per 1,000 person-years). Of these, 1,269 cases were included in NDR solely because of blood glucose measurements (without results of those tests or confirmed diabetes in other registers), and were excluded for a more strict definition of diabetes, resulting in 3,035 (5.8%) strict diabetes cases (incidence rate 5.9 per 1,000 person-years).

Diabetes cases were older at enrollment; had higher BMI, waist circumference and higher alcohol and saturated fat intake; had lower education and socio-economic status; consumed less fruit and vegetables; and were more likely to be men, unemployed, current or previous smokers, exposed to environmental tobacco smoke, be physically inactive and were exposed to similar arsenic concentrations as compared to the whole cohort. Tap water was the major source of water intake with a median intake of 1.6 L per day at enrollment (Table 1). Geographical variation of arsenic concentrations in Denmark is depicted in Figure 1. Water utilities in the Aarhus area distribute water with higher arsenic concentrations to consumers than those in the Copenhagen area. When considering the distribution of time-weighted arsenic concentrations since 1971 according to area of enrollment, concentrations amongst persons enrolled in Aarhus were higher with a median of 2.11 µg/L as compared to a median of 0.58 µg/L in Copenhagen (Figure 2).

Overall the adjusted IRR for all diabetes cases associated with each µg/L increment increase in average arsenic levels was (IRR = 1.03; 95% CI: 1.01, 1.06), whilst the adjusted IRR for strict diabetes was (IRR = 1.02; 95% CI: 0.99, 1.05). There was no exposure-dependence over the four arsenic exposure quartiles (Table 2) and we found no significant effect modifications (Table 3). Figure 3 shows increasing IRR for all diabetes with increase in time-weighted arsenic exposure

at the address since 1971, based on the fully adjusted models; for the strict definition of diabetes this relationship showed an inverse U-shape in the lower exposure range and a slightly increasing IRR at the higher concentrations; confidence intervals were wide.

Discussion

The risk of diabetes was positively associated with long-term exposure to low-level arsenic in drinking water.

This prospective cohort study relates low-level arsenic exposure in tap water used for drinking, traced to each individual address to incidence of diabetes assessed objectively from a nationwide register, where information on a wide range of potential confounding factors was collected at enrollment without potential for recall bias. Previous studies of low-to-moderate arsenic exposure and diabetes risk used self-reports of diabetes and/or physician records of fasting blood glucose/75 g oral glucose tolerance test (Coronado-Gonzalez et al. 2007; James et al. 2013; Jovanovic et al. 2013; Kim et al. 2013; Li et al. 2013; Navas-Acien et al. 2008; Navas-Acien et al. 2009; Steinmaus et al. 2009; Wang et al. 2007; Zierold et al. 2004). In this cohort we relied on records of incident diabetes during follow-up in the NDR and did not use information such as self-reports or physician records of fasting blood glucose/75 g oral glucose tolerance test used in previous studies. The use of objective measures of diabetes based on a nationwide register, NDR, is attractive since the entire population is covered by uniform inclusion and objective criteria and the drop-out rate is zero (Carstensen et al. 2008). Also, all Danish citizens have free access to the health care system, thus capture of diabetes within the entire population in the registry is assumed to be relatively free of socio-economic status, which could limit study analyses in countries that do not have free healthcare access.

Among the limitations of NDR is the lack of information regarding whether the registered diabetes is type 1 or type 2. However, type 2 diabetes generally constitutes 90-95% of all diabetes in this age group; and cohort participants who reported a diagnosis with diabetes prior to enrollment were excluded. Further limitations include that the date of inclusion in the NDR register is only a proxy for the diagnosis, which was likely made some time prior to inclusion in NDR (Glumer et al. 2003). Also the NDR likely underestimates the actual diabetes burden, as people without clinical diagnoses are not included.

Information on the level of blood glucose or other clinical measurements used at diagnosis is not available in NDR. The "all diabetes" definition of incidence in NDR has previously been validated by a study comparing register-identified patients with their general practitioners, indicating that the sensitivity and predictive value of the criteria used in NDR was above 86% (Carstensen et al. 2008). However, the definition of incidence used in NDR is based on four inclusion criteria, three of these; hospital discharge diagnoses, chiropody and diabetes medication records; reflect highly likely diabetes cases (strict diabetes cases), but the fourth regarding blood glucose measurements may lead to inclusion of non-diabetic persons. There is no information available on the results of the glucose tests, or other records in NDR for the persons included solely on the basis of blood glucose measurements. Without this information it is not possible to confirm diabetes amongst these persons, although the positive predictive value was 86% and 95% for 5 measurements in 1-year and 2 measurements per year in 5-year periods in a validation exercise, respectively (Carstensen et al. 2008). Some of these people may not have diabetes, as there is an increasing awareness among physicians in Denmark on detecting undiagnosed diabetes. Thus it is common for elderly healthy people to have five blood glucose tests per year. To account for this, we included a second strict definition of diabetes in which we

excluded the 1,269 cases of diabetes that were based solely on blood glucose tests. The results of analyses considering strict diabetes were slightly weaker than those including all diabetes in this population, possibly questioning causality of the relationship.

This prospective cohort study relates diabetes risk to individual estimates of arsenic exposure in tap water used for drinking water by consumers based on geographical and registry linkage. The inclusion of the residential address history of the 53,931 eligible participants in as many as 135,601 individual homes, over a period of 35 years adds valuable strength to this study as type 2 diabetes develops over many years (Genuth et al. 2003) and is often diagnosed years after actual onset (Glumer et al. 2003). One other very recent prospective case-cohort study including 141 cases and 488 participants also relates diabetes risk to arsenic in drinking water based on lifetime reconstruction of exposure through structured interviews, geospatial modelling of groundwater inorganic arsenic concentrations (James et al. 2013). That study also reports increased risk associated with individual exposure and although that study is smaller than the present study the authors include validation of estimated exposure using urinary inorganic arsenic species concentrations. The use of validation is a clear strength when compared to the present study or other previous studies that used average arsenic in the area as a proxy of exposure, with no information on individual exposure histories (Islam et al. 2012; Jovanovic et al. 2013; Makris et al. 2012), which would imply some exposure misclassification. The limitations of the exposure assessment used in the present study also include that the residential histories of the cohort participants, prior to 1971 were unknown, thus we could not assess the impact of early life arsenic exposure. The unknown address histories prior to 1971 may imply some exposure misclassification, due to different migration patterns prior to 1971 for cases and non-cases. In addition we assumed that arsenic measurements taken after 1987 were

representative of historical exposure, which would inherently be associated with some exposure misclassification.

In the present study we included no information regarding exposure to organic arsenic via the diet (fish, shellfish, rice and wine), but this exposure is less harmful. Fish and shellfish are considered the most important sources of organic arsenic in the diet and although fish and shellfish are included in the Danish diet, levels of consumption are low in a worldwide perspective when compared to countries such as Japan or central Asia. Also we do not expect the typical Danish diet to include much of other high arsenic foods such as seaweed. Addressing the potential effect of arsenic in seafood was not feasible, as this adjustment would likely have led to other positive associations between seafood intake per se and diabetes risk as recently discussed in a comprehensive systematic review (Zhang et al. 2013).

Finally, our estimation of arsenic exposure is based on the concentration in tap water at home measured at the water utilities outlets providing drinking water to consumers, and although residential histories are accounted for, we cannot account for temporary migration and water sources at work in other regions in Denmark or whilst overseas in areas with either higher or lower levels of arsenic in groundwater. Measurement of arsenic in nails, hair or urine would provide more precise estimates of personal exposure but was not feasible in the present study. Measurement of arsenic in urine has previously been used in several studies, but most of these considered areas of high level arsenic exposure. Amongst the studies considering moderate arsenic exposure two report significant positive associations between moderate level arsenic exposure and diabetes risk (Coronado-Gonzalez et al. 2007; Navas-Acien et al. 2008; Navas-Acien et al. 2009) and another reported a non-significant increased risk (Steinmaus et al. 2009).

Our analyses of risk were adjusted for dietary intake of vegetables, fruit and other foods based on self-administered reports at enrollment, these dietary intakes would inevitably be affected by some degree of uncertainty and were only assessed at baseline. The participant's diets as well as lifestyle factors such as smoking may have changed during the study. However this misclassification would be non-differential and unrelated to either the disease or exposure.

The importance of understanding the effects of low-to-moderate level arsenic exposure in drinking water on diabetes has led to increased interest in this association within the last decade. However, whereas evidence appears consistent at high levels of exposure; risk at low levels and possible thresholds have not been well addressed. The median arsenic exposure level at enrollment in the present study was 0.7 µg/L, which is well below the Danish guideline of 5 µg/L (DME 2007) and World Health Organization guidelines of 10 µg/L (WHO 2006) and comparable to the concentrations found in other northern European countries such as Finland (median of 0.14 µg/L) as well as USA (mean of 2 µg/L) (ATSDR 2007). Our results indicate that exposure to low-level arsenic may play a small role in the diabetes epidemic and that risk could increase by 2-3% for a 1-µg/L increase in arsenic in drinking water.

Conclusion

In a large prospective cohort study, we found a weak positive association between low-level arsenic concentrations in drinking water and the risk of diabetes. More work is needed to elucidate the role of low-level arsenic in the present diabetes epidemic.

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Table 1. Characteristics of the cohort by incident diabetes status at follow-up, for two definitions of diabetes based on the Danish National Diabetes Register [N (%) or median (5^{th}, 95^{th} percentile).

Characteristic	Total cohort	All diabetes[a]	Strict diabetes[a]
Population (N)	52,931 (100)	4,304 (100)	3,035 (100)
Age (years)	56.1 (50.7, 64.2)	57.1 (50.9, 64.4)	57.0 (50.8, 64.5)
Males	25,102 (47.4)	2,438 (56.6)	1,807 (59.5)
Body mass index BMI (kg/m^2)	25.5 (20.4, 33.2)	28.5 (22.3, 38.0)	29.1 (22.8, 38.8)
Underweight (BMI<18.5 kg/m^2)	444 (0.8)	13 (0.3)	9 (0.3)
Normal weight (18.5 ≤ BMI < 25 kg/m^2)	23,013 (43.5)	814 (18.9)	436 (14.4)
Overweight (25 ≤ BMI < 30 kg/m^2)	22,040 (41.6)	1,880 (43.7)	1,324 (43.6)
Obese (BMI ≥ 30 kg/m^2)	7,434 (14.0)	1,597 (37.1)	1,266 (41.7)
Waist circumference (cm)	88 (69, 110)	98 (75, 121)	100 (78, 122)
Length of education (years)			
< 8	17,245 (32.6)	1,699 (39.5)	1,260 (41.5)
8-10	24,468 (46.2)	1,923 (44.7)	1,330 (43.8)
> 10	11,218 (21.2)	682 (15.9)	445 (14.7)
Occupational status			
Employed	41,614 (78.6)	3,144 (73.0)	2,185 (72.0)
Unemployed/retired	11,317 (21.4)	1,160 (27.0)	850 (28.0)
Socio-economic status[b]			
Low	7,514 (14.2)	690 (16.0)	585 (19.3)
Low/medium	24,214 (45.8)	1,902 (44.2)	1,366 (45.0)
Medium/high	9,814 (18.5)	783 (18.2)	594 (19.6)
High	11,389 (21.5)	929 (21.6)	492 (16.1)
Smoking			
Never	18,790 (35.5)	1,274 (29.6)	861 (28.4)
Previous	14,874 (28.1)	1,316 (30.6)	904 (29.8)
Current	19,267 (36.4)	1,714 (39.8)	1,270 (41.8)
Environmental tobacco smoke	33,809 (63.9)	2,944 (68.4)	2,126 (70.1)
Diet			
Fruit intake (g/day)	145 (22.9, 472)	143 (19.7, 463)	138 (18.6, 454)
Vegetable intake (g/day)	158 (48.1, 351)	140 (41.8, 332)	136 (39.8, 324)
Saturated fat intake (g/day)	31.1 (15.8, 55.2)	31.4 (15.7, 56.4)	31.7 (16.3, 57.4)
Total intake of tap water (L/day)[c]	1.63 (0.70, 2.90)	1.60 (0.60, 2.80)	1.60 (0.56, 2.81)
Consume alcohol	49,948 (94.4)	3,984 (92.6)	2,794 (92.1)
Cumulative alcohol use (g)[d]	14.1 (1.31, 65.3)	14.7 (1.00, 76.4)	15.0 (1.00, 80.1)
Activity			
Physically active or play sports in leisure time	28,754 (54.3)	1,900 (44.1)	1,243 (41.0)
Physical activity (h/week)[e]	2.0 (0.5, 7.0)	2.0 (0.5, 6.0)	2.0 (0.5, 6.0)
Arsenic at baseline (µg/L)	0.70 (0.05, 2.11)	0.70 (0.05, 2.11)	0.70 (0.43, 2.11)

[a]All diabetes: based on original inclusion in the Danish National Diabetes Register: hospital admissions for diabetes, diabetes medication, reimbursement for chiropody due to diabetes, or glucose blood tests; and Strict diabetes: excluding cases that were based solely on blood tests. [b]Based on work market affiliation, income and education standards for the municipal each person lived in at enrollment. [c]Total sum of tap water, coffee, tea and fruit syrup/cordial diluted with tap water which was reported at enrollment. [d]Based on all alcohol drinkers. [e]Based on physically active/sports in leisure time participants.

Table 2. Association between arsenic exposure and diabetes among 52,931 DCH cohort participants for two definitions of diabetes incidence based on the National Diabetes Register.

Diabetes definition	Cases, n	Incidence rate ratios (95% CI) Crude[a,b]	Incidence rate ratios (95% CI) Adjusted model[a,b,c]
All diabetes[d]			
< 0.57	1049	1.00	1.00
0.57 – 0.74	1021	1.09 (1.00, 1.19)	0.96 (0.87, 1.05)
0.74 – 1.82	1017	1.06 (0.97, 1.15)	1.08 (0.99, 1.18)
> 1.82	1217	1.20 (1.10, 1.30)	1.19 (1.09, 1.31)
Linear trend per µg/L	4304	1.02 (1.01, 1.04)	1.03 (1.01, 1.06)
Strict diabetes[d]			
< 0.57	749	1.00	1.00
0.57 – 0.74	856	1.28 (1.16, 1.41)	1.03 (0.92, 1.14)
0.74 – 1.82	648	0.94 (0.84, 1.04)	1.00 (0.89, 1.11)
> 1.82	782	1.07 (0.97, 1.19)	0.99 (0.89, 1.11)
Linear trend per µg/L	3035	1.00 (0.98, 1.03)	1.02 (0.99, 1.05)

[a]Adjusted for age by using it as time scale in the Cox model. [b]Due to exclusion of cohort members with missing value in any covariate, the number of persons is identical in the crude and the adjusted analyses. [c]Adjusted for calendar year, sex, BMI (kg/m^2), waist circumference (cm), smoking (indicator: never, former, current), environmental tobacco smoke (indicator: yes, no), physical sports and activity in leisure time (indicator: yes, no and intensity (h/week)), alcohol consumption (indicator: yes/no and g/day), fruit consumption (g/day), vegetable consumption (g/day), saturated fat consumption (g/day), educational level (indicator: <8, 8-10, >10 years), socio-economic status (indicator: (low, medium low, medium high and high). [d]All diabetes: based on original inclusion in the National Diabetes Register: hospital admissions for diabetes, diabetes medication, reimbursement for chiropody due to diabetes, or glucose blood tests; strict diabetes: exclusion of diabetes cases that were based solely on blood tests.

Table 3. Modifications of associations between time-weighted average arsenic exposure[a] (per µg/L) and all diabetes cases (n = 4303) among the 52,931 DCH cohort participants.

Potential effect modifier	Cases, n	Incidence rate ratio (95% CI)[b]	P[c]
Sex			
Males	2,438	1.02 (0.99, 1.05)	0.11
Females	1,866	1.05 (1.02, 1.09)	
Length of education (years)			
< 8	1,699	1.05 (1.02, 1.08)	0.53
≥ 8	2,605	1.04 (1.01, 1.07)	
Smoking status			
Never	1,274	1.01 (0.96, 1.06)	0.62
Previous/current	3,030	1.02 (0.99, 1.05)	
Physical activity			
Yes	1,900	1.02 (1.01, 1.06)	0.98
No	2,404	1.03 (1.00, 1.06)	
Waist circumference (cm)[d]			
Low	1,007	1.02 (0.99, 1.05)	0.15
High	3,297	1.06 (0.99, 1.05)	
Cardiovascular disease[e]			
Yes	223	1.07 (0.97, 1.13)	0.69
No	4,081	1.03 (1.01, 1.05)	
Enrollment clinic			
Copenhagen	2137	0.99 (0.90, 1.09)	0.43
Aarhus	898	1.03 (1.00, 1.06)	

[a]Arsenic exposure was entered as a continuous variable in all models as the time-weighted average concentration (µg/L) in tap water at residences from 1 January 1971 until censoring. [b]We adjusted the analysis for age (underlying time-scale), calendar year, sex (indicator: males, females), BMI (kg/m^2), waist circumference (cm), smoking (indicator: never, former, present), environmental tobacco smoke (indicator: yes/no), physical sports and activity in leisure time (indicator: yes/no and intensity (h/week)), alcohol consumption (indicator: yes/no and g/day), fruit consumption (g/day), vegetable consumption (g/day), saturated fat consumption (g/day), educational level (indicator: <8, 8-10, >10 years), socio-economic status (indicator: low, low-medium, medium-high, high). However, with no adjustment for the modification variable. IRR expressed per µg/L of arsenic exposure. [c]Test of the null hypothesis that the linear trends are identical, for Wald test for interaction. [d]High waist circumference was defined as waist circumference > 102 cm in men and > 88 cm in women according to Lee and co-workers (Lee et al. 2012). [e]Including stroke and/or myocardial infarction at enrollment.

Figure legends

Figure 1. Arsenic concentrations for each utility in Denmark were calculated based on 4,954 compulsory measurements taken in 2,487 water utilities (1984-2004).

Figure 2. Distribution of time-weighted average concentrations of arsenic from 1971 to the end of follow-up stratified according to enrollment area of cohort participants

Figure 3. Spline functions (filled lines; 95% CIs indicated by dashed lines) between diabetes and average arsenic concentration at residences from 1971 onwards, based on fully adjusted models and cohort participants with exposure between the 1st and 99th percentile.

Figure 1

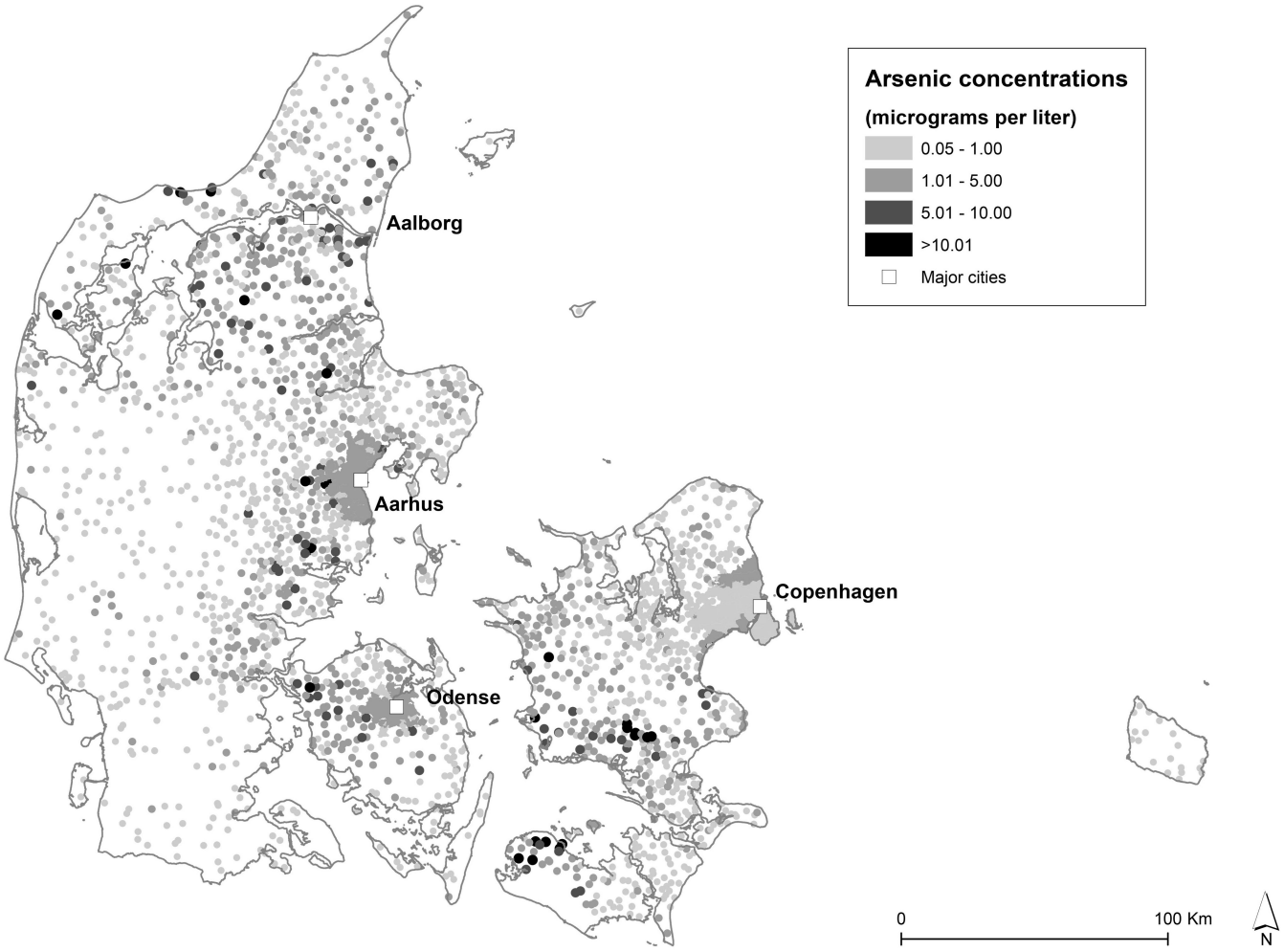

Arsenic concentrations
(micrograms per liter)
0.05 - 1.00
1.01 - 5.00
5.01 - 10.00
>10.01
Major cities
Aalborg
Aarhus
Odense
Copenhagen
0
100 Km
N

Figure 2

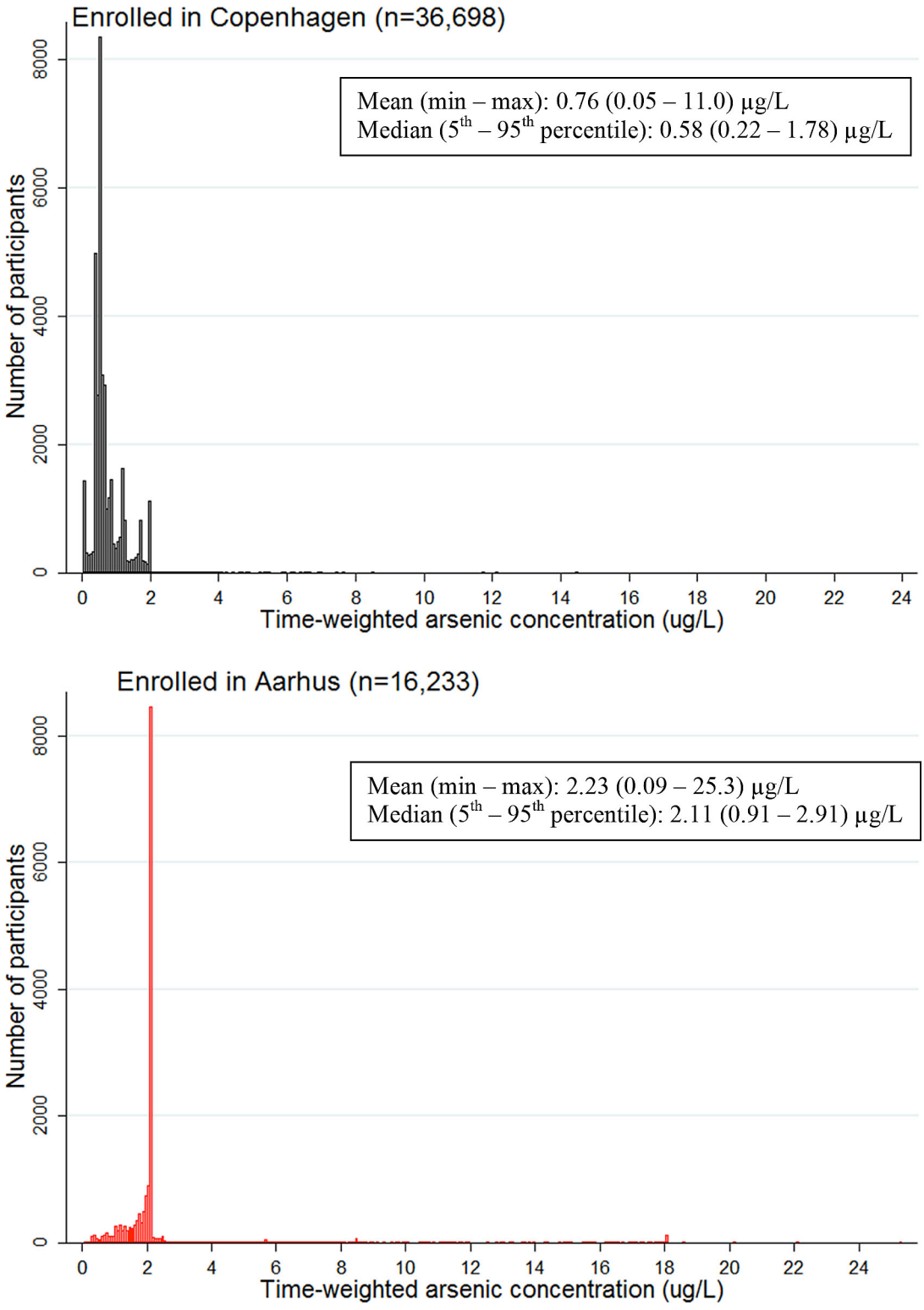
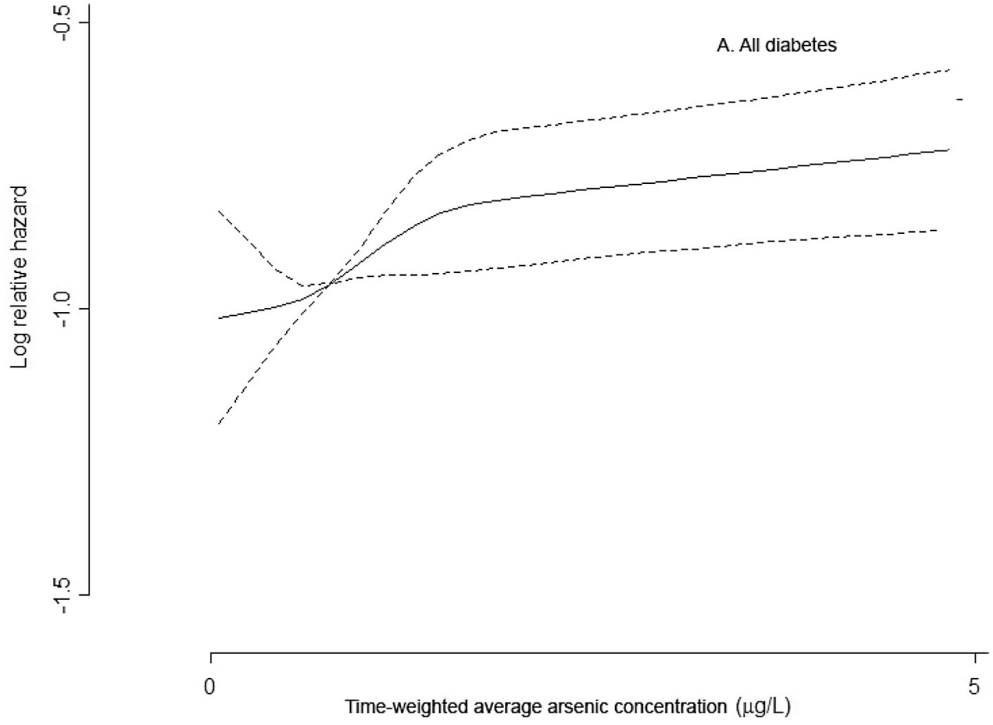
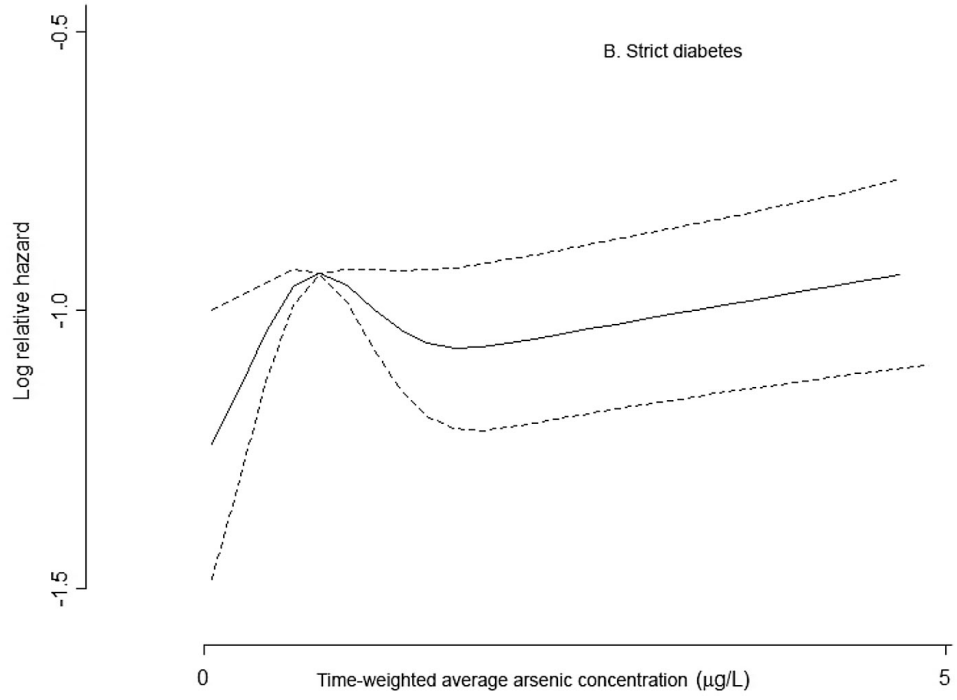

Enrolled in Copenhagen (n=36,698)
Mean (min – max): 0.76 (0.05 – 11.0) µg/L
Median (5th – 95th percentile): 0.58 (0.22 – 1.78) µg/L
Number of participants
0
2000
4000
6000
8000
0
2
4
6
8
10
12
14
16
18
20
22
24
Time-weighted arsenic concentration (ug/L)
Enrolled in Aarhus (n=16,233)
Mean (min – max): 2.23 (0.09 – 25.3) µg/L
Median (5th – 95th percentile): 2.11 (0.91 – 2.91) µg/L
Number of participants
0
2000
4000
6000
8000
0
2
4
6
8
10
12
14
16
18
20
22
24
Time-weighted arsenic concentration (ug/L)

Figure 3


A. All diabetes
Log relative hazard
-0.5
-1.0
-1.5
0
5
Time-weighted average arsenic concentration (µg/L)


B. Strict diabetes
Log relative hazard
-0.5
-1.0
-1.5
0
5
Time-weighted average arsenic concentration (µg/L)